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Genetic and maternal effects on tail spine and body length in the invasive spiny water flea (*Bythotrephes longimanus*)

Andrea L. J. Miehls,[1,2] Scott D. Peacor[1] and Andrew G. McAdam[3]

1 Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA
2 NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, MI, USA
3 Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

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Correspondence
Andrea L. J. Miehls, Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824, USA.
Tel.: +517 353 7981;
fax: 517-432-1699;
e-mail: jaegeran@msu.edu

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Abstract

Interest in the evolution of invasive species has grown in recent years, yet few studies have investigated sources of variation in invasive species traits experiencing natural selection. The spiny water flea, *Bythotrephes longimanus*, is an invasive zooplankton in the Great Lakes that exhibits seasonal changes in tail spine and body length consistent with natural selection. Evolution of *Bythotrephes* traits, however, depends on the presence and magnitude of quantitative genetic variation, which could change within or across years. Clonal analysis of wild-captured *Bythotrephes* indicated that variance components for distal spine length were variable among but not within years. Spine length was always heritable but was not always influenced by maternal effects. In contrast, variance components for body length varied both within and among years, but likewise body length was always heritable and not always influenced by maternal effects. Results indicate that important *Bythotrephes* traits have heritable variation comparable to native species and other invasive species that would enable an evolutionary response to natural selection. This evolutionary capacity could contribute to the widespread success and dramatic effects of *Bythotrephes* invasion in systems with diverse biotic and abiotic conditions.

Introduction

Invasive species are considered one of the leading threats to biodiversity and ecosystem function. Whereas the myriad and often severely negative ecological effects of species invasions have garnered considerable research attention for decades (reviewed in, e.g. Elton 1958; Mack et al. 2000; Pimentel et al. 2000; Ehrenfeld 2010), research on the evolution of invasive species has intensified only recently (reviewed in, e.g. Mooney and Cleland 2001; Lee 2002; Cox 2004; Lambrinos 2004; Strayer et al. 2006). This growing body of research suggests invasive species often evolve upon introduction into new environments because of novel selection pressures, population bottlenecks, founder effects and hybridization with related species in the invaded range, as well as freedom from selection pressures in the native range (Lambrinos 2004; Bossdorf et al. 2005). Further, there is growing evidence that evolution of invasive species may play a key role in their pronounced ecological effects (Mooney and Cleland 2001; Lambrinos 2004; Strayer et al. 2006).

Although we are beginning to appreciate the importance of evolution to the ecological effects of invasive species, we still have limited knowledge of the rate of and constraints on invasive species evolution (but see Lavergne and Molofsky 2007; Dlugosch and Parker 2008; Colautti et al. 2010). In particular, we know little about genetic variation in invasive species traits despite its potential to constrain evolutionary rates (Lynch and Walsh 1998; Conner and Hartl 2004; but see Blows and Hoffman 2005). A wealth of studies of neutral (molecular) genetic variation in invasive species have been published (Dlugosch and Parker 2008), but there have been few studies of genetic variation in quantitative traits (reviewed in Table 1; see also Dlugosch and Parker 2008). Furthermore, the few studies that have addressed quantitative genetic variation suggest no consistent pattern in the amount of genetic variation in invasive species traits;

Table 1. Measures of quantitative genetic variation in invasive species traits, including genetic and additive genetic variation (V_g and V_a, respectively), coefficients of additive genetic variation (CV_a), and broad- and narrow-sense heritabilities (H^2 and h^2, respectively).

Species	Trait	V_g	V_a	CV_a	H^2	h^2	Reference
Anolis cristatellus	Number of circum-trunk scales*					**0.27**	Eales et al. (2010)
	Number of ventral scales*					**0.48**	Eales et al. (2010)
Eurytemora affinis	Survival to metamorphosis: 15 PSU†				*0.79*		Lee et al. (2003)
	Survival to metamorphosis: 0 PSU†,‡				**1.40**		Lee et al. (2007)
	Survival to metamorphosis: 5 PSU†,‡				**0.54**		Lee et al. (2007)
	Survival to metamorphosis: 25 PSU†,‡				**0.46**		Lee et al. (2007)
Leptinotarsa decemlineata	Development time: 17°C§			*5.50*	**0.55**		Boman et al. (2008)
	Development time: 23°C§			*7.11*	**0.52**		Boman et al. (2008)
	Female adult weight: 17°C			*5.60*	0.23		Boman et al. (2008)
	Male adult weight: 17°C			*8.65*	**0.62**		Boman et al. (2008)
	Female adult weight: 23°C			*14.64*	**0.55**		Boman et al. (2008)
	Male adult weight: 23°C			*4.23*	0.07		Boman et al. (2008)
	Emergence body mass¶		0.01×10^{-3}			0.05	Piiroinen et al. (2011)
	Overwintering body mass¶		0.05×10^{-3}			0.12	Piiroinen et al. (2011)
	Metabolic rate¶		0.05×10^{-3}			0.06	Piiroinen et al. (2011)
	Mass independent metabolic rate¶		0.05×10^{-3}			0.08	Piiroinen et al. (2011)
	Diapause behaviour¶		0.08×10^{-3}			0.02	Piiroinen et al. (2011)
	Overwintering survival¶		0.17×10^{-3}			0.05	Piiroinen et al. (2011)
	Days until entered diapause¶		1.69×10^{-3}			0.16	Piiroinen et al. (2011)
Mahonia genus	Seedling growth	0.01					Ross et al. (2009)
Phalaris arundinacea	Relative growth rate**				**0.27**		Lavergne and Molofsky (2007)
	Tillering rate**				**0.47**		Lavergne and Molofsky (2007)
	Leaf number**				**0.27**		Lavergne and Molofsky (2007)
	Stem height**				**0.49**		Lavergne and Molofsky (2007)
	Root/shoot ratio**				**0.27**		Lavergne and Molofsky (2007)
	Below ground biomass**				**0.26**		Lavergne and Molofsky (2007)
	Above ground biomass**				**0.30**		Lavergne and Molofsky (2007)
	Emergence time**				**0.08**		Lavergne and Molofsky (2007)
Poecilia reticulata	Male body area			*11.79*		**0.87**	Brooks and Endler (2001a)
	Male tail area			*13.89*		**0.41**	Brooks and Endler (2001a)
	Male black area			*35.58*		**0.43**	Brooks and Endler (2001a)
	Male fuzzy black area			*55.29*		**0.79**	Brooks and Endler (2001a)
	Male iridescent area			*36.76*		**0.45**	Brooks and Endler (2001a)
	Male orange area			*67.33*		**0.96**	Brooks and Endler (2001a)
	Male orange chroma			*17.83*		0.15	Brooks and Endler (2001a)
	Male orange brightness			*20.58*		**0.47**	Brooks and Endler (2001a)
	Male total spot number			*23.67*		**0.79**	Brooks and Endler (2001a)
	Male mean brightness			*4.53*		**0.58**	Brooks and Endler (2001a)
	Male brightness contrast			*15.46*		**0.54**	Brooks and Endler (2001a)
	Male mean chroma			*1.95*		**0.55**	Brooks and Endler (2001a)
	Male colour contrast			*9.53*		**0.38**	Brooks and Endler (2001a)
	Female responsiveness to males					**0.27**	Brooks and Endler (2001b)

Table 1. Continued

Species	Trait	V_g	V_a	CV_a	H^2	h^2	Reference
	Female discrimination of males					0.11	Brooks and Endler (2001b)
	Female preference for male traits††					0.02	Brooks and Endler (2001b)
Potamopyrgus antipodarum	Size at first reproduction‡‡	*0.74*			*0.09*		Dybdahl and Kane (2005)
	Age at first reproduction‡‡	*58.59*			*0.07*		Dybdahl and Kane (2005)
	Total offspring‡‡	*47.76*			*0.20*		Dybdahl and Kane (2005)
	Reproduction rate‡‡	*0.22*			*0.12*		Dybdahl and Kane (2005)
Thymallus thymallus	Length at termination§§					*0.07*	Koskinen et al. (2002)
	Yolk-sac volume§§					*0.04*	Koskinen et al. (2002)
	Growth rate§§					*0.21*	Koskinen et al. (2002)
	Survival§§					*0.12*	Koskinen et al. (2002)
	Incubation time§§					*0.37*	Koskinen et al. (2002)
	Swim-up length§§					*0.25*	Koskinen et al. (2002)
	Hatching length§§					*0.10*	Koskinen et al. (2002)

Statistically significant values are presented in bold; values reported without reference to statistical significance are presented in italics. We obtained values from a literature search using the ISI Web of Science® with the key words 'invasive species' and 'genetic variation'. Only studies reporting univariate estimates of quantitative genetic trait variation of invasive species in their invaded range were included.
*Eales et al. (2010) derived h^2 from mother-offspring regression, and thus values may be inflated due to maternal effects.
†Only traits for invasive populations of *E. affinis* from Lee et al. (2003, 2007) were included.
‡H^2 obtained by doubling intra-class correlations reported in Lee et al. (2007).
§CV_a and H^2 averaged across invasive populations in Boman et al. (2008).
¶V_a and h^2 were calculated from V_{sire}, V_{dam}, V_{potato} and V_{resid} reported in Piiroinen et al. (2011) following Falconer and Mackay (1996); only values for both males and females were included.
**H^2 determined from Figure 5 of Lavergne and Molofsky (2007) for invasive range.
††h^2 averaged across female preferences for the following male traits reported in Brooks and Endler (2001b): attractiveness, predicted attractiveness, body size, tail area, black area, fuzzy black area, orange area, orange chroma, orange brightness, iridescent area, spot number, mean brightness, brightness contrast, mean chroma, and colour contrast.
‡‡V_g and H^2 averaged across 12 and 15°C temperature treatments in Dybdahl and Kane (2005).
§§h^2 averaged across populations in Koskinen et al. (2002).

quantitative genetic variation ranged from very low to exceptionally high (Table 1).

The amount of genetic variation for a given trait can vary in space and time owing to genotype-by-environment interactions (Pigliucci 2001) or the erosion of genetic variation by natural selection. For example, previous research with clonally reproducing zooplankton suggests that genetic variation, as well as the ratio of genetic variation to total phenotypic variation (i.e. heritability), can decline throughout the growing season as a result of clonal selection (Lynch 1984; Pfrender and Lynch 2000). Thus, to understand how genetic variation affects the evolution and persistence of invasive species, it is important that genetic variation be quantified across a range of environmental conditions across the growing season.

Recent species introductions into the North American Great Lakes offer a strong opportunity to investigate sources of variation in invasive species traits and their ramification for ecological interactions. One invasive species considered to have potent ecological effects in the Great Lakes is *Bythotrephes longimanus*, the spiny water flea (hereafter *Bythotrephes*; Fig. 1), a predatory zooplankton native to Eurasia that was introduced to North America in the 1980s (Mills et al. 1993). The invasion of

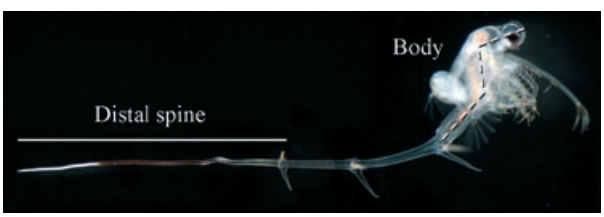

Figure 1 *Bythotrephes longimanus*, the spiny water flea. The *Bythotrephes* tail spine is comprised of the distal spine (measured from the tip of the spine to the anterior end of the first set of instar barbs; solid line) and up to two intercalary segments (for asexually produced *Bythotrephes*). Body length was measured from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax and abdomen (dashed line). The *Bythotrephes* pictured is a third instar.

Bythotrephes has resulted in dramatic ecosystem changes owing to their central position in the food web as a dominant predator of zooplankton (Bunnell et al. 2011) and common prey for fish (Pothoven et al. 2007). The primary ecological effects of *Bythotrephes* involve alteration of zooplankton community abundance and composition (Lehman 1991; Yan et al. 2002; Barbiero and Tuchman 2004), which may affect phytoplankton communities (Strecker and Arnott 2008) and larval fish (Hoffman et al. 2001) through trophic cascades.

In this study, we used clonal analysis of wild-captured *Bythotrephes* raised across three generations in captivity to measure quantitative genetic variation in two *Bythotrephes* traits, namely their distal spine and body length, which affect their susceptibility to fish predators (see Study species, below) and thus govern their food web interactions. We assessed whether genetic variation in *Bythotrephes* traits differed among five time periods across 3 years. Although the initial *Bythotrephes* invasion may have involved a genetic bottleneck (based on analysis of neutral genetic variation; Colautti et al. 2005), their rapid spread soon after invasion (Mills et al. 1993) as well as evidence for multiple introductions and gene flow among introduced populations (Colautti et al. 2005) all suggest genetic variation in the *Bythotrephes* population may be high. We therefore predicted *Bythotrephes* traits would contain significant genetic variation. Following previous studies of clonal selection in other cladoceran zooplankton (e.g. *Daphnia*; Lynch 1984; Pfrender and Lynch 2000), we further predicted that genetic variation would decline throughout the growing season.

Materials and methods

Study species

Bythotrephes reproduce by cyclic parthenogenesis and have a short generation time (10–15 day, depending on water temperature; Branstrator 2005). They produce multiple asexual generations during the growing season, which culminate in sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001; Branstrator 2005). A conspicuous trait of *Bythotrephes* is their long tail spine (Fig. 1). The longest component of the tail spine is the distal spine (i.e. spine section from the tip of the spine to the first set of instar barbs), which is present at birth and does not change with instar development (i.e. the length of the distal segment does not grow). Instead, during the first two molts, total spine length increases through the addition of intercalary segments that each end with a paired barb (Branstrator 2005).

A large volume of research documenting fish predation on *Bythotrephes* indicates that fish are size-specific predators and suggests that natural selection on *Bythotrephes* traits may be occurring, although no study has explicitly measured natural selection. *Bythotrephes* are consumed by both gape-limited fish (typically juvenile fish <100 mm length) and non-gape-limited fish (typically adult fish; Schneeberger 1991; Mills et al. 1992; Branstrator 2005; Pothoven et al. 2007), with gape-limited predation dominating later in the growing season (Branstrator 2005). Because gape-limited fish selectively choose prey based on prey size, late-season gape-limited fish predation may be an important source of selection on the size of *Bythotrephes* morphological traits. Field surveys showing a correlation between *Bythotrephes* spine and body size and the abundance of gape-limited fish (Straile and Hälbich 2000), diet analysis of wild-caught fish (Schneeberger 1991; Mills et al. 1992; Barnhisel and Harvey 1995), variable spine and body size of *Bythotrephes* among lakes with different fish predation regimes (Bilkovic and Lehman 1997; Sullivan and Lehman 1998), and evidence for selective feeding of fish on *Bythotrephes* based on size of their morphological traits (Barnhisel 1991a,b) all suggest that fish may act as agents of natural selection on *Bythotrephes* morphological traits.

Field collections

We collected *Bythotrephes* from Lake Michigan during 2007, 2008, and 2010 at either the 60-m (2007, 2008; sampling coordinates: 43°13.80′N 86°29.58′W) or 45-m (2010; sampling coordinates: 43°12.40′N 86°27.06′W) depth contours, approximately 12.5 and 9.5 km, respectively, west of Muskegon, MI. There was a single collection of 60 *Bythotrephes* mid-growing season (September 27) in 2007, three collections of approximately 300 *Bythotrephes* each spanning the growing season (July 28, September 22, and November 3) during 2008, and a single collection of 25 *Bythotrephes* early in the growing season (July 28) during 2010. Because *Bythotrephes* resting eggs hatch only at the start of the growing season (Yurista 1992) and Lake Michigan is well mixed at our sampling locations (Beletsky et al. 2007), we assumed that our samples at different time periods represented repeated samples of the same population. We used a conical zooplankton net with a 1-m-diameter opening and 363-μm mesh size towed vertically through the top 25 m of the water column. On shipboard, *Bythotrephes* were placed into 60-mL clear glass jars (one per jar) within 10 min of collection. Jars contained approximately 50 mL of Lake Michigan water previously passed through a 63-μm sieve.

Laboratory culturing

We reared *Bythotrephes* in biological incubators using methods derived from Kim and Yan (2010) and personal

communications (K. Schulz, State University of New York – College of Environmental Science and Forestry, Syracuse, New York, USA). We maintained *Bythotrephes* at 15°C with a 13:11 light/dark cycle in Lake Michigan water passed through GF/F Whatman filters. Water changes occurred every-other-day in 2007 and daily in 2008 and 2010. Each day we fed *Bythotrephes ad libitum* with approximately 150 *Artemia* nauplii that were <48 h old; in 2010, *Bythotrephes* were also given three *Daphnia pulicaria* daily.

Clonal lines

We used clonal lines (Lynch and Walsh 1998) to raise *Bythotrephes* through the F_2 generation (Fig. 2). We initiated each clonal line with a wild-captured *Bythotrephes*; the young (F_1 generation) of the wild *Bythotrephes* were used to establish clonal sublines. We assumed all wild-captured *Bythotrephes* were genetically distinct, whereas we assumed all young born to a clonal line were genetically identical (Lynch and Walsh 1998). All young born in the laboratory were transferred to individual culturing jars within 24 h of birth. In 2007 and 2008, F_2 *Bythotrephes* were reared until they reproduced or died and preserved in 95% ethanol, whereas in 2010, F_2 *Bythotrephes* were preserved at birth (first instar). Consequently, in 2007 and 2008, F_2 *Bythotrephes* were preserved at various instar stages (1, 2, and 3). Whereas distal spine length does not change through instar development, body size increases with each instar (Burkhardt 1994), so we accounted for instar stage in the body length analysis. Two of our sampling periods, September 2007 and November 2008, lacked sufficient replication of instars among clonal and subclonal lines to estimate sources of phenotypic variation. Thus, only body length data in July and September 2008 and July 2010 were analysed. However, clonal analyses of distal spine length were performed for all time periods (i.e. September 2007, July through November 2008, and July 2010).

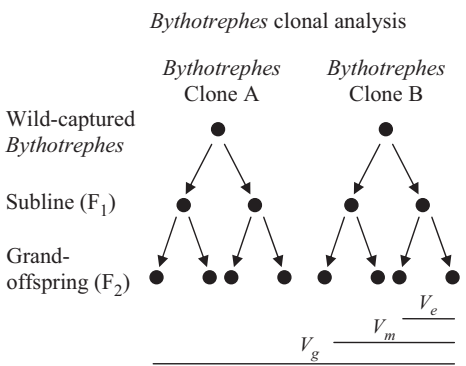

Figure 2 Clonal analysis design, with clonal sublines (F_1 generation) nested within clonal lines (wild-caught *Bythotrephes*); all analyses were completed using morphological data from the F_2 generation. V_g is the genetic variance, represented by the variance in morphology of F_2 individuals among clonal lines, V_m is maternal variance, represented by the variance among sublines within clones, and V_e is the residual environmental variance within sublines. Although two F_1 young per clonal line and two F_2 young per subline are pictured, all young born to clonal and sublines were reared. Figure modified from the study by Lynch and Walsh (1998).

Morphological measurement

We photographed F_2 *Bythotrephes* using a digital camera attached to a dissecting microscope. *Bythotrephes* were oriented on their side such that the length of the body and tail spine was along a single plane of focus and the eye, thorax and abdomen regions of the body were visible (Fig. 1). Using IMAGEJ (Abramoff et al. 2004), we measured distal spine and body length to the nearest 0.001 mm of F_2 *Bythotrephes* from the digital photographs. Whereas our measurement of distal spine length (measured from the tip of the spine to the anterior end of the first set of instar barbs; Fig. 1) was standard for *Bythotrephes* research (e.g. Sullivan and Lehman 1998), our measurement of body length was not. We noted that some *Bythotrephes* had a bend at the junction of the thorax and abdomen (as in Fig. 1), whereas others did not. Differences in body position could therefore inflate measurement variation of body length based on previously used measurement techniques (linear distance from the eye to the base of the tail spine, e.g. Sullivan and Lehman 1998). As a result, we measured body length from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax and abdomen (Fig. 1). Although some organisms are known to decrease in length after preservation in ethanol (e.g. larval fish; Moku et al. 2004), our test of *Bythotrephes* morphological shrinkage in 95% ethanol revealed no significant shrinkage of *Bythotrephes* distal spine or body length (Data S1).

Heritability, maternal effects and statistical analyses

Using our clonal breeding design, we quantified genetic, maternal and environmental variance components for distal spine and body length from statistical models of among clonal line, among subline, and within subline variation, respectively (Lynch 1985; Lynch and Walsh 1998; Fig. 2). We fitted linear mixed effects models (LME) for each trait separately (i.e. distal spine or body length) using the NLME package (Pinheiro et al. 2009) in

R version 2.12.0 (R Development Core Team 2010). In each case, the trait measured in F_2 offspring was modelled by random effects for clonal line and subline nested within clonal line. We assessed the significance of the random effects in two ways: (i) by obtaining 95% confidence intervals around the random effects through bootstrapping (Potvin and Roff 1993) and (ii) through model comparisons using likelihood ratio tests (LRT). 95% confidence intervals were obtained by randomly resampling our data set of distal spine and body lengths (1000 iterations, accounting for clonal and subline structuring), which created a distribution of variances around our random effects. For the LRT, we fitted two additional models for each trait, with each model containing successively fewer random effects. The first additional model for each trait contained the random effect for clonal line but not the random effect for subline. The second additional model for each trait was a linear model without either random effect. As *Bythotrephes* body size increases during instar development, we included instar as a fixed effect (categorical for instars 1, 2 and 3) in models of body length. The only fixed effect in models of distal spine length was the intercept.

We also tested for temporal differences in genetic and maternal variance components by assessing differences by month within 2008 (July, September, and November) and assessing differences across years. We compared a model containing time period (either month or year) as a fixed effect and all random effects described earlier to a model containing the same fixed and random effects, but which allowed clonal and subline variation to differ by time period using the varIdent function in the NLME package (Pinheiro and Bates 2000). We used a LRT to assess whether separate estimates of clonal and subline variation for each time period significantly improved the fit of the model. It is noted that this approach differs from simply assessing the significance of time period as a fixed effect, which would assess whether mean phenotypes (i.e. distal spine or body length) differ among time periods. In our analysis, we were testing whether time period affected the clonal line and subline random effects in the model.

Descendants of clonal organisms are effectively linkage groups for their entire genotype and, therefore, broad-sense heritability (H^2) is the appropriate measure of inheritance (Lynch and Walsh 1998; Conner and Hartl 2004). We estimated genetic (V_g), maternal (V_m) and environmental (V_e) variation from the variance among clonal lines, variance among clonal sublines and variance within clonal sublines, respectively, from our mixed-effect model analysis of our clonal breeding design. Based on Houle (1992), we estimated the coefficient of genetic variation (CV_g) as:

$$CV_g = \frac{100 \times \sqrt{V_g}}{\text{mean}},$$

accounting for clonal and subline structure in the trait mean. We used the variance components to calculate H^2 as:

$$H^2 = \frac{V_g}{V_g + V_m + V_e}.$$

Similar to the calculation of H^2, we calculated maternal effects (m^2) as the ratio of maternal variance (V_m) to total phenotypic variance ($V_g + V_m + V_e$).

Results

We obtained 13 clonal lines that produced F_2 *Bythotrephes* in September 2007, 18, 31, and 4 clonal lines for July, September, and November 2008, respectively, and 17 clonal lines for July 2010 (percent survival of clonal lines to the F_2 generation during the five time periods was 22, 6, 9, 2, and 68%, respectively).

F_2 *Bythotrephes* mean (±1 SD; controlled for clonal and subline) distal spine length was 4.72 ± 0.73 mm in September 2007, 5.11 ± 0.50 mm in 2008 (all months combined) and 5.70 ± 0.34 mm in July 2010. Distal spine length in 2010 was significantly greater than in 2007 ($P < 0.001$) and 2008 ($P < 0.001$); distal spine length in 2008 was also significantly greater than in 2007 ($P < 0.05$). Variance components for *Bythotrephes* distal spine length did not differ among months within 2008 (LRT: $\chi^2_2 = 3.3$, $P > 0.05$) but differed across years (LRT: $\chi^2_2 = 74.5$, $P < 0.001$). We found significant (based on 95% confidence intervals and the LRT) genetic variation in *Bythotrephes* distal spine length in 2007, 2008 and 2010 (Table 2; Fig. 3), corresponding to CV_g estimates of 15.0, 8.1, and 4.1 respectively, and H^2 estimates of 0.76, 0.48 and 0.27, respectively (Table 2). We found significant maternal variation in distal spine length in 2008 and 2010, corresponding to m^2 estimates of 0.13 and 0.59, respectively, but not in 2007 (Table 2; Fig. 3).

F_2 *Bythotrephes* mean body length for instars one, two and three was 1.53 ± 0.18 mm, 1.79 ± 0.24 mm, and 2.34 ± 0.15 mm in July 2008 and 1.61 ± 0.29 mm, 1.86 ± 0.24 mm, and 2.21 ± 0.34 mm in September 2008, respectively, and 1.58 ± 0.10 mm for instar one in July 2010. Body length for instars one, two and three did not differ by time period ($P > 0.05$ for all comparisons). In contrast to distal spine length, variance components for body length differed between months within 2008 (LRT: $\chi^2_1 = 13.4$, $P < 0.001$) and also across years (LRT:

Table 2. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components, coefficient of genetic variation (CV_g), broad-sense heritability (H^2), and maternal effects (m^2) for distal spine and body length of *Bythotrephes longimanus*.

		95% CI				95% CI			95% CI			
Time period	V_g	Lower	Upper	CV_g	V_m	Lower	Upper	V_e	Lower	Upper	H^2	m^2
Distal spine length												
2007 September	**0.498**	0.150	0.928	15.0	0.057	0.005	0.080	0.097	0.059	0.158	0.76	0.09
2008 Combined	**0.172**	0.085	0.260	8.1	**0.046**	0.011	0.122	0.138	0.109	0.176	0.48	0.13
2010 July	**0.055**	0.041	0.174	4.1	**0.118**	0.076	0.166	0.028	0.022	0.036	0.27	0.59
Body length												
2008 July	**0.016**	0.004	0.063	7.8	**0.012**	0.003	0.042	0.025	0.017	0.036	0.30	0.23
2008 September	**0.056**	0.028	0.109	13.4	<0.001	0	0.013	0.060	0.044	0.080	0.48	0
2010 July	**0.003**	0.001	0.013	3.5	**0.012**	0.005	0.022	0.012	0.009	0.015	0.13	0.44

Variance components for distal spine length did not differ by month within 2008, but differed among years. Variance components for body length differed by month in 2008 and between years. Statistically significant ($P < 0.05$) genetic and maternal variance components based on likelihood ratio tests are presented in bold; 95% confidence intervals around the variance components are given.

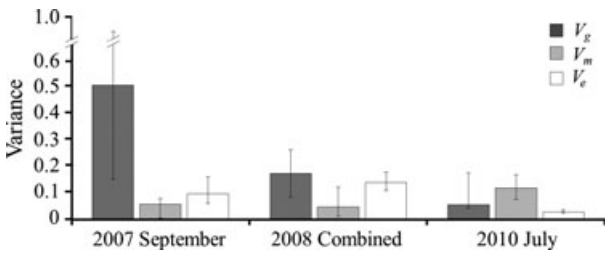

Figure 3 Genetic (V_g), maternal (V_m) and environmental (V_e) variance components for *Bythotrephes* distal spine length. Variance components did not differ by month within 2008 but differed among years. Bars are the 95% confidence intervals around the variance components.

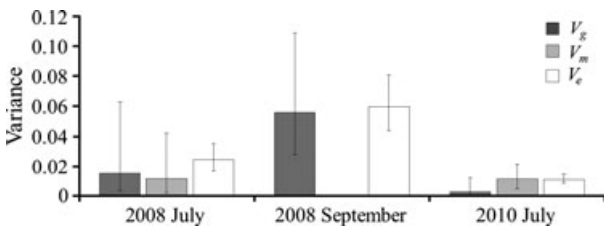

Figure 4 Genetic (V_g), maternal (V_m) and environmental (V_e) variance components for *Bythotrephes* body length. Variance components differed by month in 2008 and between years. Bars are the 95% confidence intervals around the variance components.

$\chi^2_2 = 72.5$, $P < 0.001$). We found significant genetic variation in *Bythotrephes* body length in July 2008, September 2008 and July 2010 (Table 2; Fig. 4), corresponding to CV_g estimates of 7.8, 13.4 and 3.5, respectively, and H^2 estimates of 0.30, 0.48 and 0.13, respectively (Table 2). We found significant maternal variation in body length in July 2008 and July 2010, corresponding to m^2 estimates of 0.23 and 0.44, respectively, but not in September 2008 (Table 2; Fig. 4).

Discussion

A growing body of research suggests that evolution in traits important to interspecific interactions of invasive species may play a key role in their effects on ecosystems (e.g. Mooney and Cleland 2001; Lambrinos 2004; Strayer et al. 2006). Our research quantified genetic variation in traits of the invasive zooplankton, *Bythotrephes*, a species that has caused severe and widespread ecological effects in the Great Lakes. We found that the two traits considered to have the greatest effect on food web interactions with fish, namely *Bythotrephes* distal spine and body length, both had significant genetic and maternal variation and exhibited moderate-to-high coefficients of genetic variation, broad-sense heritabilities and maternal effects (Table 2). Moreover, genetic variation for *Bythotrephes* traits did not decline over the growing season, as is typical for cladoceran zooplankton, but instead remained similar for distal spine length and increased for body length (Table 2).

Coefficients of genetic variation and heritability estimates for *Bythotrephes* distal spine and body length are similar to other invasive species (Table 1), and heritability estimates fall within the range of previously published broad-sense heritabilities for native cladoceran zooplankton, suggesting that evolution of these important *Bythotrephes* traits may not be limited by low genetic variation. Our range in heritability estimates (Table 2) for *Bythotrephes* distal spine and body length is similar to the range of heritabilities (0.28–0.62; Pfrender and Lynch 2000) for body size of *Daphnia pulex*, a widespread native *Daphnia* species. The concordance of heritability estimates between the invasive *Bythotrephes* and native *Daphnia* is significant

given that previous studies have suggested that low genetic variation relative to other sources of variation may prohibit evolution of invasive species. Multiple source populations have already been suggested for the *Bythotrephes* invasion based on evidence from neutral loci (Colautti et al. 2005) and may explain the presence of substantial genetic variation relative to other sources of variation in the traits we investigated.

Two potential sources of bias to our study must be considered. First, our heritability estimates were derived from a clonal analysis in the laboratory, which is typical for heritability estimates for cladocerans (e.g. Lynch 1984; Lynch and Walsh 1998; Pfrender and Lynch 2000), but which could introduce a bias due to experimentally controlled environmental conditions (Conner et al. 2003; Wolff 2003; Calisi and Bentley 2009; but see, Weigensberg and Roff 1996). Our estimates of environmental variation (Table 2) were consistently lower than genetic variation for *Bythotrephes* distal spine length, but similar to genetic variation for *Bythotrephes* body length and maternal variation for both traits. It is possible that low environmental variation in the laboratory inflated our estimates of heritability and maternal effects as compared to wild populations. Second, our analyses involved low sample sizes that resulted from the, at times, poor survival of clonal lines and logistical constraints of the labour-intensive culturing of *Bythotrephes* (Kim and Yan 2010), which necessitated lower sample sizes than studies of other organisms (e.g. *Daphnia*). It is possible that poor survival could have reduced our estimates of genetic and maternal variation if significant laboratory-based clonal selection occurred. However, there was no relationship between the magnitude of genetic or maternal variation and clonal line mortality; our two time periods with the lowest clonal line mortality, September 2007 and July 2010, represented the highest and lowest estimates of genetic variation in distal spine length. The lack of relationship between mortality and sources of variation indicates that mortality was random with respect to our clonal lines. Further, the absence of a significant difference in genetic variation by time period within 2008 for distal spine length was unlikely to be due to low statistical power, as these data were sufficient for detecting significant changes in genetic variation in distal spine length across years and changes in genetic variation for body length both within and across years. Thus, despite variable clonal line survival among time periods, we did not have any evidence for clonal selection associated with our culturing approaches and the remaining samples were sufficient to test our predictions.

Our findings did not support the prediction that genetic variation in *Bythotrephes* traits would decrease throughout the growing season, despite the similarity in life history between *Bythotrephes* and the other cladoceran zooplankton on which we based this prediction. Like most cladoceran species, *Bythotrephes* reproduce by cyclic parthenogenesis (i.e. multiple rounds of asexual reproduction culminating in sexual reproduction that produces resting eggs at the end of the growing season; Yurista 1992; Pothoven et al. 2001; Branstrator 2005). After sexual reproduction, genetic variation and broad-sense heritabilities for cladoceran traits are often high (with heritabilities falling within the range of 0.4–0.6) as each resting egg hatchling is genetically unique (Lynch 1984). During subsequent asexual reproduction, genetic variation typically is reduced and broad-sense heritabilities decline as a result of clonal selection, approaching zero at the end of the season (Lynch 1984). Contrary to the scenario of clonal selection eroding genetic variation over the growing season, genetic variation for *Bythotrephes* distal spine length did not differ during three sampling periods within one growing season (July–November 2008). Furthermore, genetic variation in *Bythotrephes* body length increased between July and September 2008. The increase of genetic variation in *Bythotrephes* body length was associated with a decrease in maternal variation that together resulted in increased heritability, despite a concomitant increase in environmental variation.

Multiple scenarios could explain why seasonal patterns in *Bythotrephes* genetic variation are dissimilar to other cladoceran zooplankton. Previous research documenting declines in genetic variation of *Daphnia* clones during the growing season (Lynch 1984) was associated with strong and consistent natural selection. Conversely, temporal fluctuation in the direction of selection across the growing season (Sasaki and Ellner 1997), such as from opposing selection pressures by gape-limited and non-gape-limited fish predators whose predation rate varies seasonally (Branstrator 2005), might maintain high levels of genetic variation in *Bythotrephes* traits both within and across seasons. Alternatively, phenotypically plastic responses to changing environmental conditions can prevent selection from eroding genetic variation (Ghalambor et al. 2007) and thus could explain the persistence of genetic variation in *Bythotrephes* traits. If clones differ in their plastic responses to the environment (i.e. if clones differ in their reaction norms), then certain clones may be favoured in one set of environments and selected against in another set, thus maintaining genetic variation (Pigliucci 2001). Indeed, Burkhardt (1994) and Pothoven et al. (2003) suggested that *Bythotrephes* body size may be phenotypically plastic, potentially varying in response to their abiotic (e.g. temperature) and biotic (e.g. predator or prey presence) environment. Although our results for genetic variation in *Bythotrephes* distal spine and body length suggest a more complex scenario than unidirectional clonal selection through the growing season, to

date, no study has estimated natural selection or quantified phenotypic plasticity experimentally in *Bythotrephes* traits. Thus, knowledge of selection and plasticity would be valuable for understanding why *Bythotrephes* distal spine and body length exhibit seasonal patterns in genetic variation that are atypical for a cyclic parthenogen.

Genetic variation in *Bythotrephes* distal spine and body length has implications for how *Bythotrephes* can respond to their food web interactions in the Great Lakes and could explain their invasive success in ecosystems with varying biotic conditions. Genetic variation allows for an evolutionary response to persistent environmental change across multiple generations (Lee and Gelembiuk 2008; Svanbäck et al. 2009). Our finding of moderate-to-high heritabilities throughout the growing season and across years suggests that *Bythotrephes* morphology could evolve in response to gape-limited fish predation, which increases throughout the growing season. Evolution of increased body size in response to gape-limited predation has previously been shown in *Ambystoma maculatum* (spotted salamander) larvae (Urban 2008) and *Daphnia* species (Spitze 1991). Moreover, *Bythotrephes* are abundant in a variety of lake environments with differing fish predator species and relative abundance of gape- versus non-gape-limited predators (Bilkovic and Lehman 1997; Sullivan and Lehman 1998; Young and Yan 2008). Concordantly, previous research has shown that distal spine and body length are variable among lakes (Sullivan and Lehman 1998). Potentially, the variability in *Bythotrephes* spine and body length represents local adaption to differing fish predation regimes, if *Bythotrephes* in other systems also maintain sufficient genetic variation to respond to selection from fish.

In addition to significant genetic variation, we also found significant maternal variation in *Bythotrephes* distal spine and body length that can affect phenotypic change between generations. The type of maternal effects we estimated are sometimes referred to as maternal identity effects (Sakwińska 2004) or intergenerational environmental effects (Schwaegerle et al. 2000). In a clonal analysis, maternal identity effects occur when sisters within a clonal line produce offspring that consistently differ despite being genetically identical and raised in a common environment. Maternal identity effects can be caused by maternal responses to small-scale environmental variation, which all experiments (even highly controlled laboratory experiments) inevitably involve (Schwaegerle et al. 2000), or by individual genetic differences caused by mutation. Because maternal identity effects have been shown to affect traits related to fitness (Sakwińska 2004), maternal identity effects may represent an important source of phenotypic variation in fitness-related traits of *Bythotrephes*.

In conclusion, our research has quantified genetic variation in two important ecological traits of an invasive species and has tested the stability of these sources of variation through time. Genetic variation in *Bythotrephes* distal spine and body length could contribute to the success of *Bythotrephes* at invading and dramatically altering ecosystems by permitting an evolutionary response to selection. This capacity for phenotypic change could explain, in part, the ability of *Bythotrephes* to invade lake ecosystems with variable biotic and abiotic conditions. As invasive species continue to threaten biodiversity worldwide, elucidating both the ecological and evolutionary foundations for their success will be critical.

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Supporting Information

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Data S1. Methods.

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